

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The paragraph that starts at page 28, line 10 has been amended to read as follows:

As described herein, the invention provides ANT and CypD fusion proteins comprising ANT or CypD polypeptides fused to an additional functional or non-functional polypeptide sequence that permits, for example by way of illustration and not limitation, detection, isolation and/or purification of the ANT and CypD fusion proteins. For instance, an additional functional polypeptide sequence may be an energy transfer molecule polypeptide as provided herein. ANT and CypD fusion proteins described herein may be detected by FRET, fluorescence, phosphorescence, bioluminescence, or chemiluminescence, and include fusion proteins that may in certain embodiments be detected, isolated and/or purified by protein-protein affinity (*e.g.*, receptor-ligand), metal affinity or charge affinity methods. In certain other embodiments the subject invention fusion proteins may be detected by specific protease cleavage of a fusion protein having a sequence that comprises a protease recognition sequence, such that the ANT and CypD polypeptides may be separable from the additional polypeptide sequence. In particularly preferred embodiments, for example, each ANT and/or CypD polypeptide sequence is fused in-frame to an energy transfer molecule polypeptide sequence. Other polypeptide sequences present in ANT and CypD fusion proteins may facilitate affinity detection and isolation of ANT and CypD polypeptides and may include, for example, poly-His or the defined antigenic peptide epitopes described in U.S. Patent No. 5,011,912 and in Hopp et al., (1988 *Bio/Technology* 6:1204) (*e.g.*, FLAG[®] epitope tag DYKDDDDK, SEQ ID NO:), or the XPRESS[™] epitope tag (DLYDDDDK, SEQ ID NO: ; Invitrogen, Carlsbad, CA). The affinity sequence may be a hexa-histidine tag as supplied, for example, by a pBAD/His (Invitrogen) or a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host. Alternatively, the affinity sequence may be a hemagglutinin (HA) tag when mammalian host cells, for

example COS-7 cells, are used. The HA tag corresponds to an antibody defined epitope derived from the influenza hemagglutinin protein (Wilson et al., 1984, *Cell* 37:767).

In the claims:

Claims 96, 104, 107 and 108 have been amended to read as follows:

92. A method of identifying an agent that alters binding of an adenine nucleotide translocator polypeptide to a cyclophilin polypeptide, comprising:

(a) contacting, in the absence and presence of a candidate agent, (i) a first isolated recombinant polypeptide comprising a cyclophilin polypeptide or variant thereof with (ii) a sample comprising a second isolated recombinant polypeptide that comprises a recombinant human adenine nucleotide translocator polypeptide or variant thereof, under conditions and for a time sufficient to permit the cyclophilin polypeptide, the adenine nucleotide translocator polypeptide and the candidate agent to interact; and

(b) comparing a level of binding of the first isolated recombinant polypeptide to the second isolated recombinant polypeptide in the absence of the candidate agent to the level of binding of the first isolated recombinant polypeptide to the second isolated recombinant polypeptide in the presence of the candidate agent, wherein a decreased level of binding in the presence of the agent indicates an agent that inhibits binding of an adenine nucleotide translocator polypeptide to a cyclophilin polypeptide and wherein an increased level of binding in the presence of the agent indicates an agent that enhances binding of an adenine nucleotide translocator polypeptide to a cyclophilin polypeptide, and therefrom identifying an agent that alters binding of an adenine nucleotide translocator polypeptide to a cyclophilin polypeptide.

93. The method of claim 92 wherein at least one of the first and second isolated recombinant polypeptides is a fusion polypeptide.

94. The method of claim 92 wherein the first isolated recombinant polypeptide comprises a human cyclophilin D polypeptide that is fused to an additional polypeptide, wherein the additional polypeptide is other than glutathione-S-transferase,

95. The method of claim 92 wherein the cyclophilin polypeptide is selected from the group consisting of human cyclophilin A, human cyclophilin B, human cyclophilin C and human Cyp-60.

96. (Amended) The method of claim 92 wherein the first isolated recombinant polypeptide comprises a cyclophilin polypeptide fused to an additional polypeptide that is selected from the group consisting of polyhistidine, polylysine, a haemagglutinin epitope tag, an ~~XPRESS™~~ DLYDDDDK [SEQ ID NO:] epitope tag, a FLAG® DYKDDDDK [SEQ ID NO:] epitope tag, a Myc epitope polypeptide, a FLASH peptide, an immunoglobulin constant region polypeptide, streptavidin, a green fluorescent protein polypeptide, an aequorin polypeptide, a glutathione-S-transferase polypeptide and a *Staphylococcus aureus* protein A polypeptide.

97. The method of claim 92 wherein the first isolated recombinant polypeptide is detectably labeled with a linked reporter group.

98. The method of claim 92 wherein the first isolated recombinant polypeptide comprises a cyclophilin polypeptide fused to an additional polypeptide that is polylysine and the second isolated recombinant polypeptide comprises a recombinant human adenine nucleotide translocator polypeptide fused to an XPRESS™ epitope tag.

99. The method of claim 98 wherein the first isolated recombinant polypeptide is detectably labeled with a linked reporter group.

100. The method of either claim 97 or claim 99 wherein the linked reporter group is selected from the group consisting of a radioactive reporter group, a

dye, an enzyme, a ligand, a receptor, a protease recognition sequence, a luminescent reporter group and a fluorescent reporter group.

101. The method of claim 92 wherein the sample which comprises the second isolated recombinant polypeptide comprises at least one isolated mitochondrion.

102. The method of claim 92 wherein the sample which comprises the second isolated recombinant polypeptide comprises at least one submitochondrial particle.

103. The method of claim 92 wherein the sample which comprises the second isolated recombinant polypeptide is immobilized on a solid support.

104. (Amended) The method of claim 92 wherein the second isolated recombinant polypeptide comprises a human adenine nucleotide translocator polypeptide or variant thereof that is fused to an additional polypeptide selected from the group consisting of polyhistidine, polylysine, a haemagglutinin epitope tag, an ~~XPRESS™~~ DLYDDDDK [SEQ ID NO:] epitope tag, a FLAG® DYKDDDDK [SEQ ID NO:] epitope tag, a Myc epitope polypeptide, a FLASH peptide, an immunoglobulin constant region polypeptide, streptavidin, a green fluorescent protein polypeptide, an aequorin polypeptide, a glutathione-S-transferase polypeptide and a *Staphylococcus aureus* protein A polypeptide.

105. The method of claim 92 wherein the step of comparing binding levels comprises detection of a detection reagent that specifically binds to at least one of the polypeptides selected from the group consisting of the first isolated recombinant polypeptide and the second isolated recombinant polypeptide.

106. The method of claim 105 wherein the detection reagent is an antibody.

107. (Amended) The method of claim 106 wherein the second isolated recombinant polypeptide comprises a human adenine nucleotide translocator polypeptide or variant thereof that is fused to a polypeptide selected from the group consisting of an ~~an~~ XPRESS™ DLYDDDDK [SEQ ID NO:] epitope tag and a FLAG® DYKDDDDK [SEQ ID NO:] epitope tag, and wherein the antibody specifically binds to at least one polypeptide selected from the group consisting of the human adenine nucleotide translocator polypeptide, the XPRESS™ epitope tag and the FLAG® epitope tag.

108. (Amended) The method of claim 92 wherein the first isolated recombinant polypeptide comprises human cyclophilin D and wherein the sample which comprises the second isolated recombinant polypeptide comprises at least one submitochondrial particle isolated from a *T. ni* cell that expresses a recombinant human adenine nucleotide translocator-3 polypeptide fused to an ~~an~~ XPRESS™ DLYDDDDK [SEQ ID NO:] epitope tag.